

WHAT IS CLAIMED IS:

1 1. A composition comprising a polypeptide comprising a SUMO protease
2 catalytic domain in a trapped proteolytic deacylation intermediate complex with its substrate.

1 2. The composition of claim 1, wherein the complexed molecules have a
2 crystalline structure.

1 3. The composition of claim 1, wherein the SUMO protease is Ulp1.

1 4. The composition of claim 3, wherein the substrate is Smt3.

1 5. The composition of claim 3, wherein the polypeptide consists of the catalytic
2 domain of Ulp1.

1 6. The composition of claim 2, wherein the complex has crystal structure
2 coordinates as shown in Table 1.

1 7. A method of forming a complex of a polypeptide comprising a catalytic
2 domain of a protease with its substrate, which method comprises:

- 3 (a) combining the protease with its substrate in a molar ratio to produce a mixture;
4 (b) adding a reducing agent capable of trapping a proteolytic deacylation intermediate
5 complex of the protease and the substrate in amount effective to trap an isolatable amount of the
6 complex; and
7 (c) adjusting the pH of the mixture to about 7.0.

1 8. The method of claim 7, wherein steps (a) and (b) are performed
2 simultaneously.

1 9. The method of claim 7, wherein the pH is adjusted to about 7.0 by dialyzing
2 the mixture.

1 10. The method of claim 9, further comprising isolating the complex after
2 dialysis.

1 11. The method of claim 9, wherein the protease is a SUMO protease.

1 12. The method of claim 7 where the protease is a polypeptide comprising a
2 catalytic domain of Ulp1

1 13. The method of claim 12 where the substrate is Smt3.

1 14. The method of claim 13 where Ulp1 and Smt3 are present in a molar ratio
2 from 1:1 to 1:5.

1 15. The method of claim 14 where Ulp1 and Smt3 are present in a molar ratio of
2 1:3.

1 16. The method of claim 7, wherein the reducing agent is sodium borohydride.

1 17. A polynucleotide comprising a sequence, wherein the sequence encodes a
2 mutant Ulp1.

1 18. The polynucleotide of claim 17, wherein the mutant Ulp1 comprises the amino
2 acid sequence shown in SEQ ID NO:2, but contains an amino acid substitution at a position selected
3 from the group consisting of 432, 448, 451, 455, 472, 474, 489, 490, 493, and 515.

1 19. A recombinant vector comprising the polynucleotide sequence of claim 17.

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1 20. The recombinant vector of claim 19, where the polynucleotide encodes a
2 mutant Ulp1 comprising an amino acid substitution at position 432, 448, 451, 455, 472, 474, 489,
3 490, 493, and/or 515.

1 21. A host cell comprising the recombinant vector of claim 19.

1 22. A host cell comprising the recombinant vector of claim 20.

1 23. A process of producing mutant Ulp1, comprising culturing the host cell of
2 claim 21 under conditions whereby the polynucleotide encoding the mutant Ulp1 is expressed.

1 24. A mutant Ulp1 polypeptide.

1 25. The polypeptide of claim 24, wherein the mutant Ulp1 comprising an amino
2 acid substitution at position 432, 448, 451, 455, 472, 474, 489, 490, 493, and/or 515 of SEQ ID NO:
3 2.

1 26. A method of identifying potential substrates of a cysteine protease by rational
2 drug design, which method comprises designing candidate substrates that would form interactions
3 with catalytic site amino acids identified from computer modeling based on the crystal structure of
4 the composition of claim 2.

1 27. The method according to claim 26, wherein the crystal structure has a catalytic
2 site having crystal structure coordinates as shown in Table 1.

1 28. The method according to claim 26, which further comprises synthesizing the
2 candidate substrate and determining whether the candidate substrate interacts with the cysteine
3 protease.

1 29. The method according to claim 26, wherein the candidate substrate is a
2 cysteine protease inhibitor.

1 30. The method of claim 26, wherein the protease is Ulp1.

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